

QUALITY CHARACTERISTICS OF INDIGENOUS FERMENTED BEVERAGE; PITO USING
Lactobacillus sake AS A STARTER CULTURE

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ABSTRACT

An indigenous fermented beverage; Pito, produced from sorghum using *Lactobacillus sake* as starter culture was investigated, characterised and its microbial quality established. Lactic acid bacteria a probiotic organism was isolated from the pito. The microbial and biochemical tests carried showed that the microbial isolate do not posses antimicrobial activity against *staphylococcus aureus*, *Escherichia coli*, and *klebsiella*. The introduction of the isolated lactic acid bacteria into the pito beverage and allowed incubation period of 0-144h showed significant variations in the quality characteristics of this indigenous dink. The optimum incubation period required to achieve high quality pito drink was established at 48hr at 28°C where even the microbial load of the fermented drink was within accepted limit.

KEYWORDS: pito, lactobacillus sake, indigenous fermented food, lactic acid bacteria, probiotic food.

INTRODUCTION

Pito is an indigenous Nigeria alcoholic drink produced from local grains such as guinea corn (*sorghum vulgare*) and sorghum (*sorghum bicolour*). Sorghum is one of the cereals cultivated in the tropical regions of sub-Saharan Africa. It is largely cultivated in northern Nigeria (Asiedu 1989). The nutritional composition of sorghum include starch 68-80 % , protein 10-15% moisture content 11-12% , fat 3%, fibre 2% and ash content 2% (Ihekoronye and Ngoddy 1985).

The indigenous alcoholic drink pito is produced locally by malting of the sorghum grains, fermentation and maturation (incubation period) (Ekundayo 1969). The microbial organisms associated with the fermentation of this indigenous alcoholic drink include *saccharomyces cerevisiae* , *saccharomyces chavelieria* and *leuconostoc meseteroides* . The local method of pito production is similar to methods used to produce other local alcoholic drinks like burukutu except increased pH from 4.2-5.2 within 24hr of fermentation and sharp decrease of pH to 3.7 after 48hr (Asiedu 1989). *Geotrichum candidum*, and lactobacillus species have been said to be responsible for souring pito. Another type is unfermented pito is available which is also an indigenous product produced in Ilorin, Nigeria mainly from plant extracts. The process of producing unfermented pito involves steeping, and boiling only. Kolawale *et al* (2007) have reported the chemical composition and microbial quality of pito. It has been observed that there was a variation in the nutrient content of pito produced using varying processing methods .The protein content of fermented and unfermented pito showed 2.5% and undetected level respectively. Most of the bacterial cultures found during the production of pito include lactic acid bacteria, which include those bacteria capable of metabolising lactose to lactic acid that lowers pH of product. The lactic acid bacteria have optimum pH3-6.8. Many lactic acid bacteria have been found to act as bacteriocin and have been in fermented pito. They include *Leuconostoc mesenteriod*, *Bacillus subtilis* , *Staphylococcus species* , *Geotrichum candidum* and *Lactobacillus species*. They species are responsible for the souring of pito. Due to consumers demand for the locally fermented beverages like pito, the bacteriocine –producing organisms are considered a potential source of biological preservatives for such local drinks. have reported that bacteriocine increases the protein content of such drinks and does not have the risks associated with common antibiotics and other chemical additives Bacteriocines have good thermal stability and can survive the thermal condition that is obtained in the fermentation units. Bacteriocines can work at lower pH and low temperature needed for pito fermentation. There is therefore the need to produce this local drink and asses its microbial quality and to optimise the nutrient content quality of this drink .This is the subject of this report.

MATERIALS AND METHODS

Sample collection: The sorghum grains used this study was purchased from Umuahia main market, Abia State Nigeria. The reagents used for the study were analytical grade reagents from BDH chemicals ltd London. The production of fermented pito: Five hundred grams of sorghum grains was weighed out into a clean 2-litre volume flask and 1500ml of distilled water was measured out and added into the flask, soaked for 48h at 27±0.5. Thereafter the water from the flask was decanted and the wet grains allowed germinating in a basket

lined with banana leaves at $30 \pm 0.5^\circ\text{C}$ for 5 days. The germinated grains now known as malted grains, then ground to slurry paste in a grinder using water and boiled at 98°C for 2.5h, allowed to cool and filtered using muslin bag. The filtrate was collected into 1500 ml flask allowed to stand overnight for fermentation to take place at $30 \pm 0.5^\circ\text{C}$.

Preparation of culture sample: One millilitre of the produced pito was pipetted out into a prepared 9ml Man Ragoza and Sharpe (MRS) broth that has already been autoclaved at $121 \pm 0.5^\circ\text{C}$ for 15mins. The sample broth was incubated for 24h.

Microbial isolation: Sterilised test tubes were arranged serially and 9ml of peptone water measured out using sterile syringe and transferred into each of the tubes. Each of the tubes covered with a sterile cotton wool and aluminium foil, autoclaved at $121 \pm 0.5^\circ\text{C}$ for 15mins. Thereafter 1ml of the culture was each transferred into each of test tubes. This gave a concentration dilution of 0.1M. A serial dilution of each test tube was carried continuously until a final concentration of each tube gave a concentration of 10^{-10} M. One millilitre from each of the 10^{-10} M was pipetted out into a sterilised Petri dish containing MRS agar meal and each test tube incubated for 24h at $30 \pm 0.5^\circ\text{C}$. The isolate from each of the test tube culture was purified by streaking culture method (Onyeagba, 2004) to obtain a pure culture isolates.

Identification of isolate tests: The isolates were examined microscopically and followed by series of biochemical tests carried out as described below;

- (i) Gram staining: A thin film of each of isolates was smeared on grease free slid and the slide content mixed with a drop of saline water. Each slide was air -dried and heat fixed by passing each slide over the blue flame of a burning Bunsen burner repeatedly. Each of the slides after the heat treatment was flooded with a crystal violet solution, allowed for 1min, washed with distilled water for 5mins. Each of the slides then stain with iodine solution, and allowed for a minute each. The stains were then removed with distilled water and repeated washing after decolourising has taken to place. Then counter staining of each of the test tubes was carried using safranin and each allowed for 30sec, washed again and air-dried, viewed under oil immersion objective lens ($\times 1000$) of the light microscope. The gram -negative organisms retain the stains whereas the gram - positive ones do not (Onyeagba, 2004).
- (ii) Catalase test: A few 2drops of prepared 3% hydrogen peroxide (H_2O_2) was added into each of the slides described in (i) and the production of gas an indication of positive test monitored (AOAC, 1990).
- (iii) Fermented sugar test: A test solution containing 10ml peptone water, 5g of NaCl, 2.5mg of phenol red was prepared in a litre of distilled water. Ten ml of this solution was measured out and 1g of sugar added, thoroughly mixed and autoclaved at $121 \pm 0.5^\circ\text{C}$ for 5mins. Sub cultured organisms from the prepared culture for 16h was inoculated into each of the ten ml solution and incubated at $30 \pm 0.5^\circ\text{C}$ for 120h. Changes in colour and gas production were monitored every 24h period for the 5days duration.(AOAC,1990).
- (iv) Arginine test: Arginine broth solution was prepared using 5g of peptone, 5g of yeast extract, 2g of K_2HPO_4 , and 500mg of glucose and 3g of arginine. Five ml each of this arginine solution was measured into a labelled test tube and freshly prepared isolate from the fermented pito was inoculated into each of the tube and incubated at $30 \pm 0.5^\circ\text{C}$ for 24h. Thereafter 0.25ml of Nessler reagent was added into each tube. The formation of arginine hydroxide characterised by the development of a brown colouration was monitored.
- (v) Motility test: Sulphur indole motility solution 1.5g of MRS medium was weighed out and added into a container containing 50ml deionised water and heated to obtain complete solution. Ten ml of this solution was measured out into a test tube autoclaved at $121 \pm 0.5^\circ\text{C}$ for 15mins and allowed to cool and isolate inoculated, incubated at $37 \pm 0.5^\circ\text{C}$ for 48hr. A diffused growth or turbidity away from the line of inoculation indicates a positive result, but growth along the inoculation line indicates a negative result (Cowan, 1974).

- (vi) Inhibition test: The test organisms were inoculation into 5ml of MRS broth and incubated at $30 \pm 0.5^\circ\text{C}$ for 18h. The pH was adjusted to 6.2 with sterile syringe in Na OH. Subsequently it was centrifuged at 300revm for 15mins and indicated into a sterile test tube to get cell-free supernatant (AOAC, 1990) .The target organisms; *E coli*, *Staphylococcus aureus*, and *Klebsiella* were grown in Macon key and nutrient agar respectively for 16h. Using a sterile 1ml syringe, to measure out 0.1ml of the 10^{-7} sample culture was added into semi- solid tryptonesoy, Macon key and nutrient agar were added subsequently. Forty millilitre of this medium prepared was each poured into sterile Petri dish and allowed to solidified. Agar wells were aseptically bored inside the solid agar and 1ml of cell –free supernatant was poured into the well and incubated for 24hr at $37 \pm 0.5^\circ\text{C}$. The inhibition zone and diameter were measured after the incubation time.
- (vii) pH measurement: One millilitre of the pito was measured out into 10ml of deionised water and stirred to form a uniform solution and the probe of the pHmeter dipped into it and the value read out (Onyeagba 2004)
- (viii) Total solid measurement: The total soluble solid was determined according to AOAC (1990) method.

$$\% \text{ TSS: } \frac{W_2 - W_1 * 100}{V_s}$$

Where w_1 is the weight of empty dish, w_2 weight of the dish plus the sample and V_s of the sample.

- (ix) Determination of the total titrable acidity: Twenty gram of the fermented pito was measured into a 250ml flask and 50ml of distilled water, the flask incubated for 40 mins and filtered using whatman filter paper. Twenty of this filter ate was titrated against 0.01M NaOH solution using phenolphthalein indicator.

$$\text{Total titrable acidity} = \frac{V_b * N_b}{20\text{ml}}$$

Where V_b is the volume of Na OH used, and N_b is the normality of the NaOH and 20ml is the volume of the pipette used.

- (x) Determination of the microbial load of the fermented pito: Serial dilution of the fermented pito was carried out using nine different test tubes and each test tube was filled with 9ml of peptone water, 1ml of the pito added in each test tube. Continuous serial dilutions of each test tube yield a final concentration of each test tube to 10^{-9}ml .
- (xi) Plating and counting: The spread plate method was used according to due Shreve and Arils (2003). An agar medium was used and the incubation carried out at $37 \pm 0.5^\circ\text{C}$ for 24hr. There after the microbial load was counted using the gallenkemp colony counter. The plate count per ml of the pito sample was calculated thus; the number of colonies on each plate CFU/ml * the dilution factor of the pito sample = plate count per ml of the sample. CFU/ml = colony forming unit divided by the dilution factor 10^{-9}ml

RESULTS AND DICUSSION

The results of the production of fermented local beverage drink pito and its quality assessments are presented in Tables 1, 2, 3, and Figures 1 and 2 respectively

Table 1: Microbial load of fermented pito with lactobacillus

Incubation period (h)	Microbial load (cfu/ml)*10 ⁻⁵
0	1.59±0.92
12	3.46±0.63
24	3.46±0.63
48	3.30±0.61
72	3.60±0.40
96	3.60±0.40
126	3.60±0.40
144	3.60±0.40

From Table 1 it was observed that there was a significant variation in the population of the micro organisms with the incubation period variations ($p < 0.05 > 0.05$). The isolated micro organisms from the fermented pito and the organisms' characteristics are showed in table 2

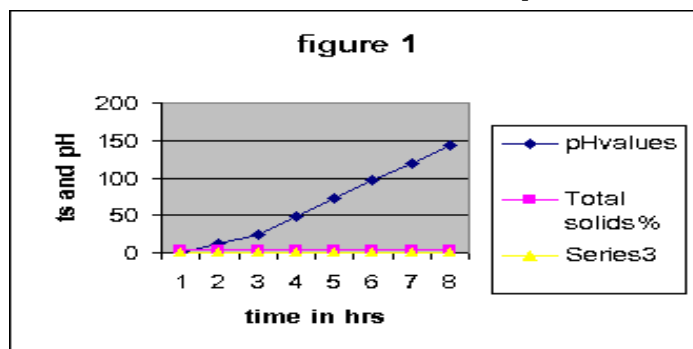
Table 2: Biochemical and morphological characteristics of isolates

Test name	respond	respond	response
Catalase	-	-	-
Gram staining	+	+	+
Morphological test	Long rods	Long rod	Long rod
Arginine test	+	+	+
Sodium chloride (4%)	+	+	+
Sodium chloride (5%)	+	+	+
Motility test	+	+	+
Inhibition test	-	-	-
Arabinose glucose	-	-	-
Lactose	+	+	+
xylose	+	+	+
Sucrose	+	+	+
Manitol	+	+	+
Raffinose	+	+	+
Maltose	+	+	+
Galactose	+	+	+
Sorbitol	+	+	+

+ =positive and acid production in sugar.

- = negative and no acid production in sugar.

Figure 1: The effect of incubation time on total solids and pH of fermented pito



The three isolates fermented all the sugars except arabinose and galactose. The three isolates did not produce gas after the 5 days fermentation. The isolates changed the colours of the test solution from red to yellow which was an indication of acid production (Okaka and Okaka 2001). This result was in accordance with works by others (Vander berg *et al* 1993) on the fermented pito with lactic acid bacteria.

Comparison with the standard showed the organism to be *Lactobacillus* and biochemical tests further confirmed it to be *Lactobacillus sake*. This organism was suspected because pure culture of *Lactobacillus sake* has been isolated from fermented pito samples (Kolawole *et al* 2007). The inhibition test indicated that the organisms did not possess antibacterial activity against *Staphylococcus aureus*, *Escheria coli* and *Klebsiella*. This result showed that it did not possess bacteriocins.

Changes in the quality parameters of pito inoculated with isolated lactic acid bacteria at different concentrations namely; (a) Total titrable acid (TTA): The result of the titrable acidity has been shown in Fig1. There was significant difference ($p < 0.05$) in the total titrable acidity of the pito drink at different incubation periods. The result showed that TTA was highest at 120h of incubation closely followed by 144h and 96h of incubation respectively. The increase in TTA agreed with earlier findings (Kolawole *et al* 2007) on fermented.

pH variation is shown in Fig1. The highest pH value was obtained at the start of the fermentation or at 0h. The variation decreased as the fermentation time duration increases. This was in agreement with similar studies carried on fermented pito drinks (Amove *et al* 2007). The total solid values obtained were shown in Fig2. The values decrease with fermentation duration. Similarly, the total soluble solids showed a reverse trend from the total solid values. These findings are in accord with earlier reports on fermented pito.

Table3: The red ox potential and temperature of fermented pito

Period of incubation in hr	Red ox potential mV	Temperature °C
0	53.0±.00	26.76±.300
12	54.8 ±.0.73	27.46±.27
24	56.26±.64	27.84±.25
48	57.06±.66	28.84±.100
72	49.76±1.57	27.92±.29
96	49.15±1.30	27.76±.23
120	47.46±0.34	26.69±.31
144	47.48±.72	27.15±.37

From Table 3 both the red ox potential and the temperature variation with fermentation time did not occur in a regular and predictable fashion

CONCLUSION

The various characteristic parameters investigated in a locally fermented pito drinks showed that this local drink of Nigeria origin can serve as a potential probiotic food for the indigenous native when hygienically produced.

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